

Flood Caused Changes to the Upper Guadalupe River Riparian Forests of Central Texas

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ABSTRACT

Pre-flood phytosociological measurements were made in eight apparently mature riparian forest communities along the upper Guadalupe River in central Texas. After a high magnitude 100+ year flood in 1978, six communities were revisited and post-flood phytosociological measurements were made to examine flood effects on the riparian forest communities. Some riparian forests were completely destroyed with woody plant trunks snapped or plants uprooted and washed downstream. We viewed these as early successional communities, and did not study them. The flood caused changes in the riparian forest communities we studied previously including reductions in community basal area and density, as well as species basal area, density, and percent cover. Overall, community species composition was unchanged based on Shannon-Weaver diversity values (H'). Species with the greatest reduction in community basal area or density were *Taxodium distichum* (Bald cypress), *Carya illinoensis* (Pecan), *Ulmus americana* (American elm), *Celtis laevigata* (Hackberry) and *Acer negundo* (Box-elder). Greatest community damage seemed to be related to tree size and bank position. Apparently, the larger the tree or species, the greater the damage sustained in the flood. Larger trees or species at the edge of the river sustained the greatest community change while the smallest ones at greater distance from the river edge had the least change. However, aerial photographic evidence of major river bends, areas that we did not study, suggested that water was so deep and the flood force so great that it cut across the bend removing most trees and vegetation in its path. Published on-line www.phytologia.org *Phytologia* 99(4): 226-237 (Dec 18, 2017). ISSN 030319430.

Key Words: basal area, community structure, density, Edward Plateau, flood damage, floods, Guadalupe River, riparian forest, woody plants.

Understanding community structure and factors that determine structure is a difficult task. Usually more than one force or factor is involved and they could be biotic, abiotic or a combination. Abiotic forces or physical forces such as fires, wind, tidal waves, volcanoes, earthquakes, floods and landslides can have major impacts on plant communities and their component species (Begon et al. 2006). Biotic forces or factors such as herbivory or competition can have similar community effects and usually more than a single force is in play (Begon et al. 2006). All of these forces or combinations can be important in directing community succession (Whitmore 1974; Putz et al. 1983; Begon et al. 2006; Van Auken and Bush 2013).

Riparian forest composition and structure has been studied for many years, including studies in central Texas (Van Auken et al. 1979; Ford and Van Auken 1982). There are spatial gradients that at least partially control the species composition of riparian forests. For example, flood tolerant trees or those requiring more water are close to the river or stream bank and those less tolerant are more distant from the water edge (Bush and Van Auken 1984). Effects of prolonged inundation on tree growth for species on

low gradient streams has been well investigated (reviewed by Hupp 1988; also see Johnson et al. 2000). Short term effects of deep, rapidly moving water on woody species and community structure are not as well understood because they occur so infrequently. Some qualitative studies of flood damage have appeared in the literature including mention of complete destruction of all trees in parts of floodplain forests, as well as uprooting, breaking and shearing of stems (Shull 1944; Gardner 1951; Eisenlohr 1952; Hack and Goodlett 1960; Lindsey et al. 1961; Sigafos 1964; Hallgren 1979; Sullivan 1983).

Recent studies in central and western North America have demonstrated specific flood effects including the importance of floating debris, logs and whole trees (Johnson et al. 2000). In addition, changes in riparian forests on ephemeral streams have been examined (Friedman and Lee 2002). Predictive theories have also been developed that consider various aspects of extreme flood events including water force, material transport, flood frequency and conditions of the floodplain or bank (Dixon et al. 2015).

Early studies of riparian communities did not have much access to or use of aerial photography and overviews of damage to large areas were not easily accomplished, but that has changed (Figure 1, Johnson et al. 2000; Friedman and Lee 2002; Dixon et al. 2015). These studies mainly examined flood effects on early establishing or early successional floodplain species and communities. It has been suggested that temperate floodplain forests are good examples of communities that show a need for chronic, patchy disturbance (Horn 1976). The area of some of these patches may be extensive and show the results of regular destruction which would appear to be cyclic and triggered by periodic high magnitude floods.

Southwestern and central Texas has one of the most varied rainfall-runoff regimes in the United States (Patton and Baker 1976). The rivers occur in bedrock canyons that are deeply entrenched in the Edwards Plateau. The hills are steep, the limestone-derived soils are shallow and the vegetation is comparatively sparse (Van Auken et al. 1979; Kochel 1988). Flood stages have been recorded between 25 and 30 m above normal water levels in some areas (Figure 2, Kochel 1988). On August 1-4, 1978, more than 120 cm of rain from a stalled weak tropical depression (Tropical Storm Amelia) fell on the watersheds of the Medina and Guadalupe Rivers in central Texas (Hallgren 1979; Sullivan 1983). Most of this rain fell during a period of 24 hours causing a major flood. There were 27 deaths in central Texas attributed to the rains and subsequent floods. Normal rainfall for this area is about 81 cm/year, but is highly variable (Arbingast et al. 1976; NOAA 2004). The return time for such a flood has been estimated to be several hundred years (Baker 1975; Hallgren 1979; Kochel 1988). Effects of the runoff on the riparian forest communities in some areas were extensive (Figure 1 and 2). Trees greater than 2 m in diameter were up-rooted or the stems were completely shattered with the stem being removed. Others 1 m in diameter were found completely bent-over, parallel to the ground, with vertical splits in the stems (Hallgren 1979; Sullivan 1983).

PURPOSE

The purpose of this study was to identify and quantify changes in the woody vegetation of the upper Guadalupe River riparian forest caused by a high magnitude flood. An additional purpose was to show that the flood caused openings in the tree canopy reduced overstory community cover. The same pre-flood and post-flood riparian communities will be examined to demonstrate the vegetation changes.

METHODS

After analyzing and comparing results of an earlier phytosociological study of the Guadalupe River riparian forest (Ford and Van Auken 1982), we felt there was considerable variability in our data and additional effort would be necessary to understand these forest communities. Consequently, we

located and sampled eight additional inner floodplain forest communities to increase our knowledge of this system. These riparian communities were relatively narrow (15-25 m) and relatively steep (slope ~ 21°) usually with agriculture land above the riparian woodland or forest.

Pre-flood sampling. During June and July 1978, phytosociological data was collected from eight riparian communities in Comal, Kendal and Kerr counties in central Texas. All study locations were above Canyon Reservoir, between approximately 50 and 75 mi north or northwest of San Antonio, Texas. We examined the distribution of species and community characteristics on the bank of the inner floodplain, between the river and the grassland or pasture using adjacent 3 x 100 m rectangular quadrats (Van Auken et al. 2005). Basal area, density, cover and species diversity (Shannon and Weaver 1949) were determined and mean values were calculated. Relative values for basal area and density were calculated but are not presented. Relative canopy cover was estimated by visual sighting along a line perpendicular to a level surface at 25 m intervals along the upper edge of each quadrat. Plant species names and identifications were determined using “Vascular plants of Texas” (Correll and Johnston 1979) and confirmed at the United States Department of Agriculture web site (USDA 2016).

Post-flood sampling. During September 1978, six of the original communities were re-sampled (after the flood). Because of community destruction and difficulties getting through the flood debris (Figure 2), a different but equivalent sampling procedure was used (Cottam and Curtis 1956). The point-centered-quarter (PCQ) procedure was used to sample each community. Twenty-five points were sampled at 10 m intervals along a transect line parallel to and approximately 5 m from the river edge. A perpendicular to the transect line was established creating four corners where the perpendicular line crossed the transect line. The point was at the intersection of the two lines. For the woody plant closest to the point in each corner, the species was identified; then, point-to-plant distance, and circumference at approximately 15 cm above ground level were measured and recorded. Only live plants were measured. Basal area, density, cover and species diversity were determined and mean values calculated. Relative values for basal area and density were calculated but not reported. Trees with sapped or broken stems were not counted or measured.

Data analysis. Significant differences between the pre-flood and post-flood communities were determined. Total community mean basal area, density, cover and diversity were compared. Statistical significance of comparisons was determined at the $P=0.05$ level using paired t -tests (Sall et al. 2012).

RESULTS

Mean total community basal area was reduced 22% (Table 1). Four of the largest species, *Taxodium distichum* (L.) Rich. (Bald cypress), *Carya illinoensis* (Wang., K. Koch., Pecan), *Ulmus americana* L. (American elm), and *Celtis laevigata* Willd. (Hackberry) had average decreases in basal area of 8-33% (Table 1). The next four species in the table also had large losses in community basal area (Table 1). The remaining species in table 1, low community basal area species, had small or no reductions in basal area.

Riparian forest community density was reduced 26% (Table 2). *Carya illinoensis*, the species with the highest pre-flood density, changed very little after the flood. Species that were found higher up on the floodplain had little or no change in mean density post-flood (*C. illinoensis* and *U. crassifolia*). Species found at the river edge had the greatest reduction in density (22-30%, Table 2). However, less common or small floodplain species had little or no change in mean density.

Species richness in the riparian communities was 31 woody species (Tables 2 and 3). Most of the species had low density and basal area. The three tree species with the largest mean species basal area in descending order were *T. distichum*, *C. illinoensis* and *U. americana* (Table 4). The largest tree measured was a *Taxodium distichum* that had a diameter of 1.15 m. Relative cover was reduced 28% by the flood (from $96\pm 4\%$ to $69\pm 5\%$, paired *t*-test, $P < 0.05$). Species diversity (H') did not change post-flood (2.06 ± 0.34 pre-flood and 2.01 ± 0.35 post-flood, paired *t*-test, $P > 0.05$).

DISCUSSION

Biotic and abiotic forces can have major effects on plant communities (Whitmore 1974; Putz et al. 1983; Begon et al. 2006; Van Auken and Bush 2013). Changes in worldwide plant communities including North American and central Texas plant communities have been going on since prehistoric times. Changes in riparian communities have also occurred in the past and will continue to change in the future (Barbour and Billings 1988; Crawley 1997; Begon et al. 2006; Van Auken and Bush 2013). A successional sequence in these various riparian communities is involved, but fire, wind and herbivory do not seem to be the forces directing the succession. In the riparian woodlands, the major forces influencing the composition and structure of the communities is flood frequency, the time between events, the magnitude of the flood, river bank position and amount of floating debris (Johnson et al. 2000; Friedman and Lee 2002; Dixon et al. 2015).

Curiously, we thought the riparian communities we studied pre-flood were mature communities based on the literature available, the species present and our understanding of these communities at that time (Ford and Van Auken 1982). The riparian communities we examined pre-flood did not include any *Salix* trees (willows) or *Populus* species (cottonwoods) and only a few *Platanus occidentalis* (sycamore). These species are all known or reported to be early successional riparian woodland species (Barbour and Billings 1988; Crawley 1997; Van Auken and Bush 1988). We have not found any studies concerning succession in the central Texas riparian forest communities. However, we speculated that *T. distichum* is an early successional species (Ford and Van Auken 1982).

Since that time, we have not found *Taxodium* juveniles in any of the riparian communities that we surveyed (Van Auken 1988, 1993). Some preliminary studies suggested that *T. distichum* is a high light requiring species (not found below the canopy), indicating that it is an early successional species (Van Auken and Taylor, unpublished). Another species found, *Celtis laevigata*, is a shade tolerant and high soil nitrogen requiring species (typically found below a canopy). Characteristics suggest it is a later successional species, one that would grow in the shade of an overstory canopy (Van Auken and Bush 1985; Bush and Van Auken 1986; Van Auken and Bush 2013) and this current study suggests that as well.

In central Texas, populations of upland species like live oak and Texas persimmon show evidence of recruitment (Van Auken et al. 1980). Conversely, other upland deciduous species like *Quercus sinuate* (Texas red oak), *Q. glaucoides* (Lacy oak), *Prunus serotina* (black cherry), *Fraxinus texensis* (Texas ash), and *Juglans nigra* (black walnut) had few or no new juveniles in the smallest size classes examined (Van Auken 1988, 1993). Therefore, there is little or no recruitment of juveniles of these species into the adult populations.

This lack of recruitment of adults occurs in both upland communities and riparian communities. Possible reasons for this lack of recruitment are changes in local climate, changes in the disturbance regime, increases in herbivore populations, few large carnivores, or episodic establishment that reflect local environmental conditions (Harper 1977; Harcombe and Marks 1978; Beschta and Ripple 2009; Van Auken and Bush 2013). Records do not indicate local climate changes, although they are occurring and will be more obvious in the future (Grunstra and Van Auken 2015). Proximate conditions may be more important such as populations of white-tailed deer in central Texas that have increased dramatically in the

last 75+ years (Doughty 1983). These large deer populations have been implicated in the lack of recruitment of various upland deciduous woody species in central Texas (Russell and Fowler 1999, 2002, 2004; Nelson-Dickerson and Van Auken 2016).

This lack of recruitment has been demonstrated for populations of *T. distichum* (Van Auken 1988), which is similar to trends from floodplain forests in the eastern United States (Sharitz et al. 1990). Reasons seem to be high light levels that are not present below a canopy but would occur in disturbances (Neufeld 1983; Titus 1990). If *T. distichum* is to remain a component in these central Texas riparian forests, disturbances of these ecosystems should continue. Populations of *C. laevigata* in these same riparian communities seem to have large numbers of seedlings and there seems to be recruitment into the adult population. *Celtis laevigata* is shade tolerant and a mature riparian forest dominant species unlike *T. distichum* that appears to be shade intolerant (Van Auken and Bush 1985).

Elevated levels of CO₂ and concomitant increases in temperature will undoubtedly effect the species in this area and the community structure in the future, but how is uncertain. Elevated levels of CO₂ have caused increased levels of photosynthesis in some species, but associated increases in temperature had no effect compared to current temperatures (Grunstra and Van Auken 2015).

Riparian communities in central Texas, like other plant communities will change with disturbances, old communities with large/old *T. distichum* will mostly be destroyed by periodic high magnitude floods (depending on river position and water depth). Thus, the age of old *T. distichum* trees or communities in this area could be used to predict the approximate time for the next high magnitude flood event or perhaps when the previous one occurred, but neither has been done. There are probably other factors to be considered when making predictions, such as sand or gravel bars, chutes, and the cut banks of curves in the river that may be extremely susceptible to flood damage (Figure 1). We found no evidence of damage to the Guadalupe River riparian forest trees due to inundation as in other river systems, perhaps because floods of central Texas Rivers seem to occur and dissipate very quickly (Baker 1975; Hallgren 1979; Sullivan 1983).

Larger trees seemed to experience the greatest damage, which suggests that they are the most susceptible to the force of the flood. The smallest species and individuals would be subject to the lowest water velocity, because of their size and position in the water column. Woody plants with small canopies would also offer the least resistance to the flood water. Debris trapping (Sigafos 1964; Johnson et al. 2000) would also be reduced because small individuals would be completely submerged and removed from the surface where the floating debris occurs. Larger trees such as mature *T. distichum* trees, were in many cases almost completely covered by the flood waters and subjected to greater water velocities, debris catching and greater stress (Figure 2, see Sigafos 1964; Johnson et al. 2000). Flood induced changes in riparian forests are difficult to assess and recovery times are uncertain. It is known that high magnitude floods do recur at regular though potentially widely-spaced intervals in this ecosystem, and are apparently an important part of its dynamics. They may even be required if recruitment of early successional species is to occur in these communities.

Recruitment of early successional species in these floodplain forests seems to require light gaps in the forest very much like the light gaps in other forests caused by senility, disease, insects, wind throw and fire (Brewer and Merritt 1978; Garwood et al. 1979; Putz et al. 1983; Begon et al. 2006; Van Auken and Bush 2013). High magnitude floods do in fact reduce cover and create gaps; they also recur at predictable, though widely-spaced, intervals in this ecosystem (Baker 1975; Kochel 1988), and are apparently an important force and part of the dynamics of this ecosystem.

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Table 1. Flood induced changes in community mean basal area (m^2/ha) for 11 of the highest basal area riparian species found in the Guadalupe River riparian forest. Scientific names, common names, pre-flood, post-flood and % changes are presented. Total pre and post-flood mean basal areas are significantly different* (paired t -test, $P < 0.05$).

SPECIES	NAME	BASAL AREA (m^2/ha)		% CHANGE
		PRE-FLOOD	POST-FLOOD	
<i>Taxodium distichum</i>	Bald cypress	61.1	44.1	-33
<i>Carya illinoensis</i>	Pecan	24.1	22.0	-8
<i>Ulmus americana</i>	American elm	3.6	2.9	-19
<i>Celtis laevigata</i>	Hackberry	3.3	2.4	-27
<i>Platanus occidentalis</i>	Sycamore	2.1	1.9	-10
<i>Morus rubra</i>	Red mulberry	1.1	0.3	-63
<i>Acer negundo</i>	Box-elder	0.9	0.8	-12
<i>Ilex decidua</i>	Possum-haw	0.6	<0.1	-86
<i>Cornus drummondii</i>	Dogwood	0.1	0.1	0
<i>Ulmus crassifolia</i>	Cedar elm	0.1	0.1	0
<i>Diospyros texana</i>	Persimmon	<0.1	<0.1	0
Others (20 species, see Table 3)		1.6	2.1	+31
Total*		98.6±19.2	76.5±18.3	Mean = -22

Table 2. Flood induced changes in community mean species density (plants/ha) for 11 of the highest density riparian species found. Pre-flood, post-flood and % changes are presented. Total pre- and post-flood mean densities are significantly different* (paired t -test, $P < 0.05$).

DENSITY (plants/ha)			
SPECIES	PRE-FLOOD	POST-FLOOD	%CHANGE
<i>Carya illinoensis</i>	229	227	-1
<i>Celtis laevigata</i>	141	103	-27
<i>Acer negundo</i>	139	96	-30
<i>Ulmus americana</i>	63	46	-27
<i>Taxodium distichum</i>	58	45	-22
<i>Cornus drummondii</i>	55	46	-26
<i>Ilex decidua</i>	43	31	-28
<i>Diospyros texana</i>	40	29	-27
<i>Platanus occidentalis</i>	13	12	-8
<i>Morus rubra</i>	15	10	-33
<i>Ulmus crassifolia</i>	12	12	0
Others (20 species, see Table 3)	328	176	-46
Total*	1124±171	833±112	Mean = -26

Table 3. Other species found in the Guadalupe River riparian forest including common names and type of woody species. These species are low basal area and low density species.

Species	Common Name	Vegetation Type
<i>Vitis mustangensis</i>	Mustang grape	Vine
<i>Sapindus drummondii</i>	Soapberry	Tree
<i>Parthenocissus quinquefolia</i>	Virginia creeper	Vine
<i>Ptelea trifoliata</i>	Skunk-bush	Shrub
<i>Fraxinus americana</i>	White ash	Tree
<i>Cephalanthus occidentalis</i>	Buttonbush	Vine
<i>Maclura pomifera</i>	Osage orange	Tree
<i>Juglans nigra</i>	Black walnut	Tree
<i>Sideroxylon lanuginosum</i>	Gum bumellia	Tree
<i>Prosopis glandulosa</i>	Honey mesquite	Tree
<i>Melia azedarach</i>	Chinaberry	Tree
<i>Toxicodendron rydbergii</i>	Western poison ivy	Vine
<i>Quercus virginiana</i>	Live oak	Tree
<i>Amorpha fruticosa</i>	Bastard indigo	Shrub
<i>Callicarpa americana</i>	American beautyberry	Shrub
<i>Juniperus asheii</i>	Ash juniper	Tree
<i>Salix nigra</i>	Black willow	Tree
<i>Ailanthus altissima</i>	Tree of heaven	Tree
<i>Smilax bona-nox</i>	Cat-brier	Vine
<i>Tilia amercana</i>	Carolina basswood	Tree

Table 4. Flood induced changes in mean species basal area (cm²/plant) for seven selected riparian species, including the four highest community total basal area species. Pre-flood, post-flood and % changes are presented.

SPECIES	MEAN BASAL AREA		
	PRE-FLOOD	POST-FLOOD	% CHANGE
<i>Taxodium distichum</i>	11,478	9,450	-18
<i>Carya illinoensis</i>	992	845	-15
<i>Ulmus americana</i>	878	938	+7
<i>Ulmus crassifolia</i>	315	271	-14
<i>Celtis laevigata</i>	245	247	+1
<i>Cornus drummondii</i>	13	11	-15
<i>Diospyros texana</i>	5	6	+20



Figure 1. Photographs of damage to the riparian forest of the Medina River. Top figure is ground level and shows the removal of the tops of all *Taxodium* trees on the river edge. The middle and bottom figures are aerial photographs showing a number of different kinds and amounts of damage to the floodplain forest. In most cases parts of the riparian forest were completely removed or destroyed by the flood. The middle picture (aerial photograph), lower, central part shows all of the *Taxodium* trees with branches removed and apparent total removal of any forest on the right side of the photograph. Bottom picture (aerial photograph), lower center, next to the river shows *Taxodium* trees down or apparently washed away as the flood waters washed over the river edge. Photographs taken from Sullivan 1983, with permission.



Figure 2. Examples of damage to *Taxodium distichum* and the riparian forest of the Guadalupe River after the 1978 Guadalupe River flood. Upper left, a mature *Taxodium* tree apparently undamaged with uprooted trees or broken stems on the bank of the river. Upper right, two mature *Taxodium* trees with tops removed. Lower left, piles of debris on the river bank with a large *Taxodium* tree standing in the approximate center with a large, broken limb caught in the upper branches approximately 16 m (63 ft.) above ground level. Lower right, large *Taxodium* tree with the two large top stems broken off and removed. Two broken or uprooted trees are at a diagonal in the photograph. Photographs were taken by the senior author.

Key to *Juniperus occidentalis* Hook. and *J. o.* forma *corbetii*.

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ABSTRACT

Juniperus occidentalis forma *corbetii* R. P. Adams can be readily distinguished by its shrubby habit and compact foliage vs. typical *J. occidentalis* plants, that have a strong central axis and loose, open foliage (Fig. 2). A key is presented to aid in the identification of *J. o.* forma *corbetii*. Published on-line www.phytologia.org *Phytologia* 99(4): 238-240 (Dec. 18, 2017). ISSN 030319430.

KEY WORDS: *Juniperus occidentalis*, *Juniperus occidentalis* forma *corbetii* R. P. Adams, Key, identification, Cupressaceae.

Juniperus occidentalis, *J. grandis* (= *J. occidentalis* var. *australis*) and *J. osteosperma* are three very closely related junipers in the western United States (Adams, 2014, Vasek, 1966). Recently, a new taxon, *J. o.* forma *corbetii* R. P. Adams was described (Adams, 2012) based on its shrubby form and compact foliage (Fig. 1,3) vs. typical *J. occidentalis* plants, that have a strong central axis and loose, open foliage (Fig. 2).



Figure 1. Mark Corbet with an extremely shrubby habit of *J. occidentalis* forma *corbetii*, 32 km east of Bend, OR (cf. Adams 11949-11951) at the type locality.

Figure 2.(right). *Juniperus occidentalis* near Sage Hen, CA, with a strong central axis, and loose, open foliage.





Figure 3. Other plants of *J. o.* forma *corbetii* with less extreme shrubby habit.

In addition, Adams (2012) reported the volatile leaf oil of *J. occidentalis* is dominated by sabinene, p-cymene, citronellol and bornyl acetate, whereas f. *corbetii* shrubs, east of Bend, OR, have larger amounts of p-cymene (20.0) and bornyl acetate (24.5%).

Principal coordinates analysis using 42 terpenoids showed (Adams, 2012) the f. *corbetii* shrubs, east of Bend, were clearly separated (see Fig. 3, Adams, 2012).

Overall, Adams (2012) found the leaf essential oils of populations of *J. occidentalis* were rather uniform except for the populations at the extremity of the range, and for the shrubby forma *corbetii*, east of Bend, OR.

For identification of f. *corbetii* from specimens, it seems most efficient to utilize the foliage density and length of ultimate branchlets. This is illustrated in Fig. 4. Notice that the foliage of *J. occidentalis* is open and individual branchlets are clearly visible (Fig. 4, left). In contrast, the foliage of f. *corbetii* is compacted, with short sub-branching (Fig. 4, right).



Figure 4. Comparison of the foliage of *J. occidentalis* and f. *corbetii*.

Key to *J. occidentalis* and f. *corbetii* :

1. Foliage loose and open, sub-branchlets elongated, trees with strong central axis.....*J. occidentalis*
1. Foliage compact and dense, sub-branchlets shortened, shrubs.....*J. o.* forma *corbetii*

ACKNOWLEDGEMENTS

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***Lycianthes textitlaniana*, a New Species from Southern Mexico**

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ABSTRACT

A new species, *Lycianthes textitlaniana* E. Dean (Solanaceae), is described from the Sierra Madre del Sur of the state of Oaxaca, Mexico. The affinities of *L. textitlaniana* are uncertain, because it differs from other known species of *Lycianthes* in its combination of glandular pubescence, long calyx appendages, and turbinate fruit with dry mesocarp. The seeds and pubescence of *L. textitlaniana* are somewhat similar to those of *L. pringlei* (B. L. Rob. & Greenm.) Bitter of western Mexico. To help with identification of this new species, *L. textitlaniana* is compared here with *L. pringlei*, *L. surotatensis* Gentry, *L. gorgonea* Bitter, *L. purpusii* (Brandeggee) Bitter, and *L. dejecta* (Fernald) Bitter, and a photograph of the seed is provided. Published on-line www.phytologia.org *Phytologia* 99(4): 241-248 (Dec. 18, 2017). ISSN 030319430.

KEY WORDS: *Lycianthes*, Solanaceae, Mexico, Oaxaca, taxonomy

Lycianthes (Dunal) Hassler (Solanaceae) is native to both the New and Old Worlds and includes 150 to 200 species (Hunziker, 2001). The majority of its taxa are distributed in the New World (from Mexico to Argentina), with ca. 40 taxa native to Mexico (ca. 15 endemic, one of them described here) (Villaseñor, 2016). The genus is the closest relative of the chile pepper genus *Capsicum* L. (Walsh & Hoot, 2001; Bohs & Olmstead, 1997). The German botanist Georg Bitter, who monographed the genus *Lycianthes*, was the first worker to point out that *Lycianthes* and *Capsicum* share a similar calyx morphology in which the five sepal lobes are truncated into a sleeve, below which may protrude five to ten appendages (commonly called calyx teeth) (Bitter, 1919). However, while *Capsicum* species have anthers that dehisce by longitudinal slits, the species of *Lycianthes* typically have poricidal anther dehiscence.

While working on species descriptions for all Mexican and Central American species of *Lycianthes*, the first author found a specimen of a new *Lycianthes* species under the name *Lycianthes gorgonea* Bitter at the Herbario Nacional, Universidad Nacional Autónoma de México (MEXU). It was immediately obvious that this was a new species due to a unique combination of characters: glandular pubescence, long calyx appendages, turbinate fruits with dry mesocarp, and seeds with deeply indented “pits” between wavy cell walls, producing an ornamented surface (Figures 1 and 2). The turbinate fruits with dry mesocarp are unlike any other known *Lycianthes*. The pubescence and seed surface somewhat resemble those of *L. pringlei*, but it differs from that species in having very long calyx appendages.

This species is only known from one locality in Mexico and a single collection made in August, 2006 by Alma Zárate-Marcos in a canyon by Colonia Nueva, to the west of the town of Santiago Textitlán, Sierra Madre del Sur, Oaxaca. Ms. Zárate-Marcos made the collection as part of a floristic project managed by one of the co-authors (Salas-Morales) in her role as Director of the “Sociedad para el Estudio de los Recursos Bióticos de Oaxaca” (SERBO). Santiago Textitlán is a community with forest management practices certified by the Forestry Stewardship Council and has been recognized for its good forest management (Blackman et al., 2015). Before this project, the flora of the region of Santiago Textitlán was undocumented. Since the initiation of the Santiago Textitlán floristic project, nearly 7500 specimens in 140 families, 517 genera and 1172 species have been collected in the region (figures from the SERBO database). In addition, several new species from this region have been described (Borhidi et al., 2008; Turner, 2013).

Although we are describing this species based on very limited material (only the type collection), the material is very complete, with flowers, mature fruits, and seeds. By describing and naming this very unusual species of *Lycianthes*, we are adding to the knowledge of the biodiversity of Oaxaca, as well as facilitating the identification of future collections of this species. Terminology here follows that of previous papers on this genus (Dean, 1998; Dean et al., 2007).

Lycianthes textitlaniana E. Dean, sp. nov. TYPE: Mexico: State of Oaxaca, Distrito Sola de Vega, Municipio Santiago Textitlán, Colonia Nueva, 1562 m, 18 Aug 2006, *Alma Zárate Marcos AZM-274* (holotype: MEXU-1229513 (Figure 1); isotype: SERO-45464).

Diagnosis. This species is unlike any other *Lycianthes* species in its combination of glandular trichomes, long calyx appendages, dry turbinate and apiculate berries lacking fleshy mesocarp, and round-edged seeds with pitted surfaces. Indument and seeds are somewhat similar to those of *L. pringlei*, but *L. pringlei* lacks calyx appendages and has ovoid fruits with fleshy mesocarp.

Shrub or vine, ca. 0.5 m tall (reported as an herb on the label of the only known collection, but stems are obviously woody). **Indument** of clear to white, multicellular, simple, glandular (glandular tip golden-yellow to grey), wavy to patent trichomes 0.25–1.5 mm long. **Stems** pale green (drying tan) when young, not much compressed when dried, becoming brown and woody with age, moderately to densely pubescent. Upper sympodial branching points a mixture of dichasial and monochasial branching (mostly monochasial), the upper sympodial units 1–5 cm long, 1–2.5 mm in diameter, difoliate, the leaves often geminate or unifoliate due to loss of small geminate leaves. **Leaves** simple, usually paired and unequal in size, the large ones with blades 3–7 × 1.5–3 cm, the smaller ones 1.5–5 × 0.5–2 cm, the leaf pairs similar in shape, the blades ovate, to elliptic, chartaceous, moderately to densely pubescent; major veins 4–5 on either side of the midvein; base truncate, cuneate, or attenuate, sometimes oblique; margin irregularly wavy; apex acuminate; petioles absent or 0.1–1 cm long. **Flowers** 5-merous, solitary, axillary, the axes moderately to densely pubescent; peduncles absent; pedicels ca. 20–25 mm long and erect in flower, 20–30 mm long and arching to deflexed in fruit; calyx 3–3.5 mm long, 3.5–4 mm in diameter, campanulate, moderately to densely puberulent, the margin truncate, with 10 spreading, linear, basally flattened appendages, 7–10 mm long emerging ca. 0.5 mm below calyx rim; fruiting calyx accrescent, widely bowl-shaped, 4–6 mm long, 9–12 mm in diameter, the appendages to 15 mm long; corolla oriented horizontally, 1.2–1.5 cm long, rotate, mostly entire (with shallow notches), with abundant interpetalar tissue, purple adaxially, color of abaxial side unknown, glabrous, open when collected, uncertain of timing of diurnal movements; stamens unequal, with four short filaments 1–2 mm long and one long filament 2–3 mm long, glabrous, anthers 3.5–4 mm long, elliptic to lanceolate, not connivent, yellow, the pores ovate, nearly terminal, dehiscing upward, not opening into longitudinal slits; ovary not examined; style linear, ca. 7 mm long, glabrous; stigma capitate, decurrent down two sides. **Fruit** a dry berry lacking juicy mesocarp, 13–15 mm long, 9–12 mm in diameter, turbinate, the tip apiculate, pale greenish orange

(possible darker) when mature, glabrous, lacking sclerotic granules; seeds ca. 50–80 per fruit, $1.75\text{--}2 \times 1.25\text{--}1.5$ mm, round-edged, laterally compressed, reniform, tan-orange, the surface pitted (Figure 2).

Distribution. *Lycianthes textitlaniana* is only known from the type collection from the state of Oaxaca, Mexico, in the District of Sola de Vega, just outside of the town of Santiago Textitlán at 1562 m in elevation (Figure 3).

Habitat. Three of the co-authors (Dean, Torres-Colín, and Hinojosa-Espinosa) attempted to locate the new species in the field on September 7–8, 2017, after obtaining permission to explore the collection area from local authorities on September 7. Despite extensive exploration of the canyons near the latitude/longitude coordinates given on the collection label, the species was not located, although another *Lycianthes* species, *L. acapulcensis* (Baillon) D'Arcy, collected by Ms. Zárate-Marcos in the same location on the same date was encountered. The vegetation of the canyons that we explored consisted of Pine-Oak forest with elements of cloud forest and palms with the following species: *Quercus peduncularis* Née, *Arbutus xalapensis* Kunth, *Oreopanax*, *Senna hirsuta* (L.) H. S. Irwin & Barneby, *Piper umbellatum* L., *Miconia glabrata* Cogn., *Psacalium cirsiifolium* (Zucc.) H. Rob & Brettell, *Bidens pilosa* L., *Podachaenium eminens* (Lag.) Sch. Bip., *Achimenes antirrhina* (DC.) C. V. Morton, *Crusea hispida* (Mill.) Rob., *Begonia gracilis* Kunth, *Hyptis capitata* Jacq., *Micropleura renifolia* Lag., *Phaseolus leptostachyus* Benth., and *Canavalia hirsuta* (M. Martens & Galeotti) Standl. The trunks of the trees in the areas had fire scars, and members of the community reported that a fire went through the area sometime after 2006. The effect of the fire on this species is not known but could be one reason that we could not locate it at the type locality.

Phenology. The type specimens, collected in mid-August, each have one flower and several mature fruits. Therefore, the species most likely begins flowering in July, near the beginning of the rainy season, and probably continues fruiting in September.

Etymology. The specific epithet honors the community of Santiago Textitlán, Oaxaca.

DISCUSSION

Lycianthes textitlaniana is unlike any other known species in the genus, and it can be easily distinguished from other Mexican species with glandular trichomes (*L. pringlei* and *L. surotatensis*) by its turbinate fruit shape and dry mesocarp (Table 1). A number of *Lycianthes* species have calyx appendages as long as those found in *L. textitlaniana*, but none of the other species with long calyx appendages also have simple, glandular trichomes and turbinate fruit. Table 1 provides the characteristics of four other species with relatively long calyx appendages for comparison (*L. surotatensis*, *L. gorgonea*, and *L. purpusii*). Turbinate fruits are found in several of the Mexican species of series *Meizonodontae* (such as *L. ciliolata* (M. Martens & Galeotti) Bitter and *L. dejecta* (Fernald) Bitter) (Bitter, 1919). The other species with turbinate fruits have eglandular trichomes. The seeds of *L. textitlaniana* are most similar to those of *L. pringlei* in having a pitted seed surface (Table 1; Dean et al., 2007). The closest relative of *L. textitlaniana* is not known.

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Figure 1. Image of holotype of *Lycianthes textitlaniana*, Alma Zárate Marcos AZM-274 (MEXU-1229513). Use of specimen courtesy of the Herbario Nacional de México, Universidad Nacional Autónoma de México.



Figure 2. Seed of *Lycianthes textitlaniana* (taken from the holotype *Alma Zárate Marcos* AZM-274 (MEXU-1229513)); scale bar equals 0.3 mm. Photograph, by D. McNair, produced by stacking 45 images taken with a 10x objective lens attached to a Nikon DSLR. Use of specimen courtesy of the Herbario Nacional de México, Universidad Nacional Autónoma de México.

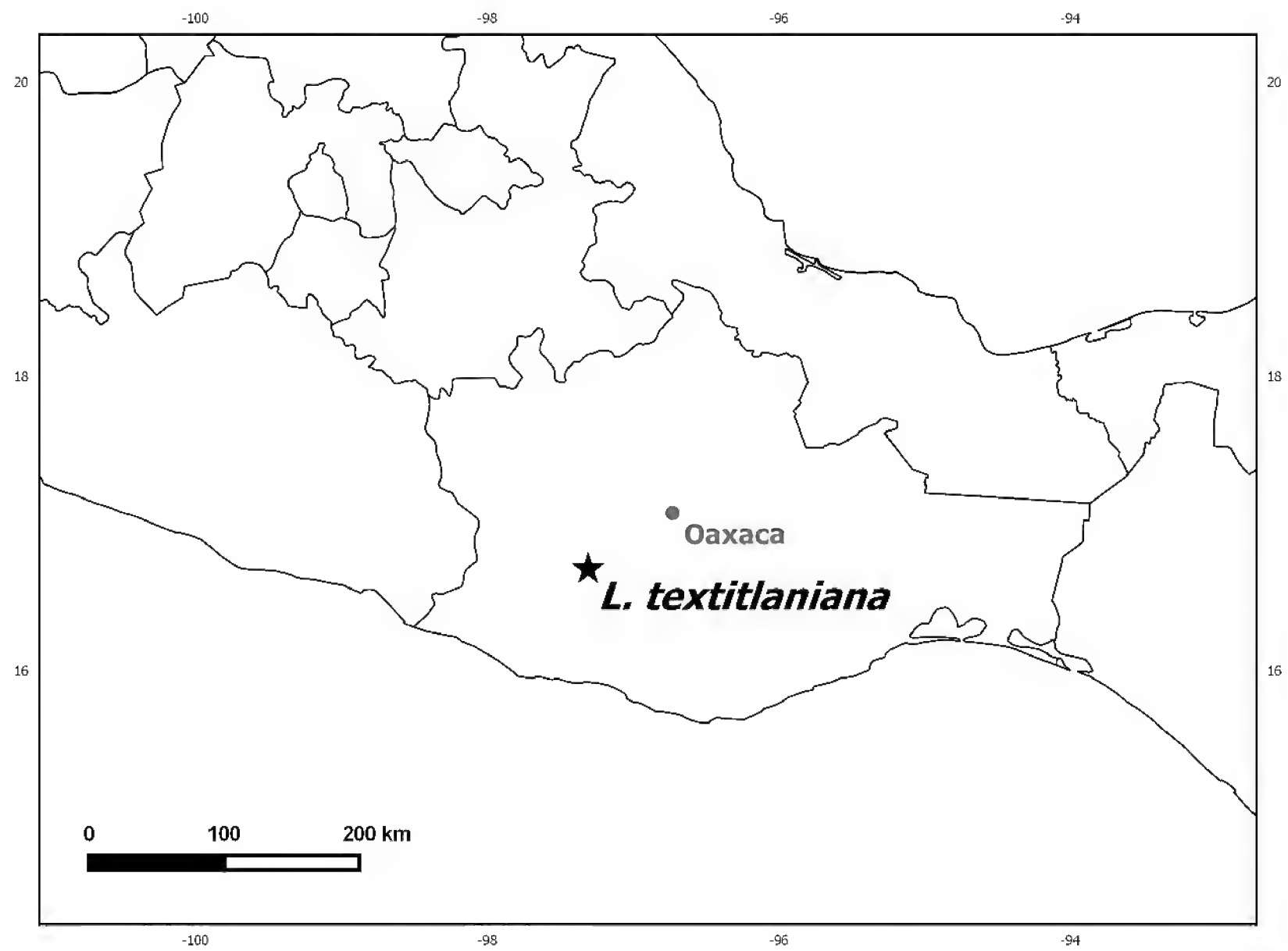


Figure 3. Map of known distribution of *Lycianthes textitlaniana*.

Table 1. Comparison of six characters in *Lycianthes textitlaniana*, *L. pringlei*, *L. surotatensis*, *L. gorgonea*, *L. purpusii*, and *L. dejecta*.

Species	textitlaniana	pringlei	surotatensis	gorgonea	purpusii	dejecta
Habit	Probably shrub or vine	Shrub	Shrub	Vine	Vine	Perennial herb
Trichome type	Simple, glandular	Simple, glandular	Simple, glandular and eglandular	Simple, eglandular	Simple to branched, eglandular	Branched to stellate, eglandular
Calyx appendage length in flower	7–10 mm long	Appendages reduced to protuberances	2–10 mm long	7–15 mm long	8–12 mm long	2–7 mm long
Fruit shape	Turbinate, tip apiculate	Ovoid, tip rounded	Spheric to depressed spheric, tip rounded	Spheric to depressed spheric, tip rounded	Spheric, tip rounded	Turbinate, tip apiculate
Mesocarp	Scant pulp, appearing dry, seeds free	Juicy pulp, surrounding seeds	Juicy pulp, surrounding seeds	Juicy pulp, surrounding seeds	Juicy pulp, surrounding seeds	Juicy pulp, surrounding seeds
Seed surface	Pitted	Pitted	Lacking pits	Lacking pits	Lacking pits	Lacking pits, fibrils on walls

Geographic variation in *Juniperus drupacea*: DNA sequencing and volatile leaf oils: Further evidence of putative Pleistocene genetic isolation between Europe and Asia

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ABSTRACT

Recently, Sobierajska et al. (2016), using nSSR and morphology, showed that *Juniperus drupacea* exhibited differentiation between Greece and Turkey/ Lebanon, suggestive of Pleistocene genetic isolation. Here, we report that leaf terpenoids and DNA sequence data support their hypothesis by confirming differentiation between Greece and Turkey/ Lebanon/ Israel. The leaf oils of the Turkey/ Lebanon plants contained one unique terpene (trans-verbenol, 0.1-1.4%) that was absent in the Greece plants. The Greece oil contained three terpenes not found in the Lebanon/ Turkey plants: (ar)-curcumene (2.2%), β -alaskene (0.3%) and α -alaskene (0.4%). Four other terpenes were in higher concentration in the Greece oils: camphene (0.4%), δ -3-carene (10.9%), p-mentha-1,5-dien-8-ol, isomer (0.3%) and 4-terpineol (0.3%). Three terpenes were higher in Turkey and Lebanon oils: α -pinene (10.5 - 32.9%), hexadecanoic acid (0.4 - 1.4%) and trans-totarol (0.3 - 1.2%). Only one SNP was found (in nrDNA) that separated Greece from Turkey-Lebanon-Israel. No informative SNPs were found in petN-psbM, trnS-trnG, trnD-trnT or trnL-trnF cp regions. Published on-line www.phytologia.org *Phytologia* 99(2): 249-257 (Dec. 18, 2017). ISSN 030319430.

KEY WORDS: *Juniperus drupacea*, Cupressaceae, geographic variation, terpenoids, nrDNA, cp DNA.

Recently, Sobierajska et al. (2016) reported on variation in nSSR and morphology for *Juniperus drupacea* Labill. from Greece, Turkey and Lebanon. They found morphology clearly separated Greece plants from those in Turkey and Lebanon (Fig. 1). In addition, they found some differentiation between plants from the Taurus (Turkey) and Lebanon mountains (Fig. 1).

Analysis using nSSR data gave a similar pattern, with some differentiation within Greece, and within Turkey populations.

Sobierajska et al. (2016) discovered a lower level of diversity in nSSR data in the Greece populations versus more diversity in Turkey and Lebanon populations. They reasoned that genetic isolation during the Pleistocene had led to the differentiation between the European and Asia populations.

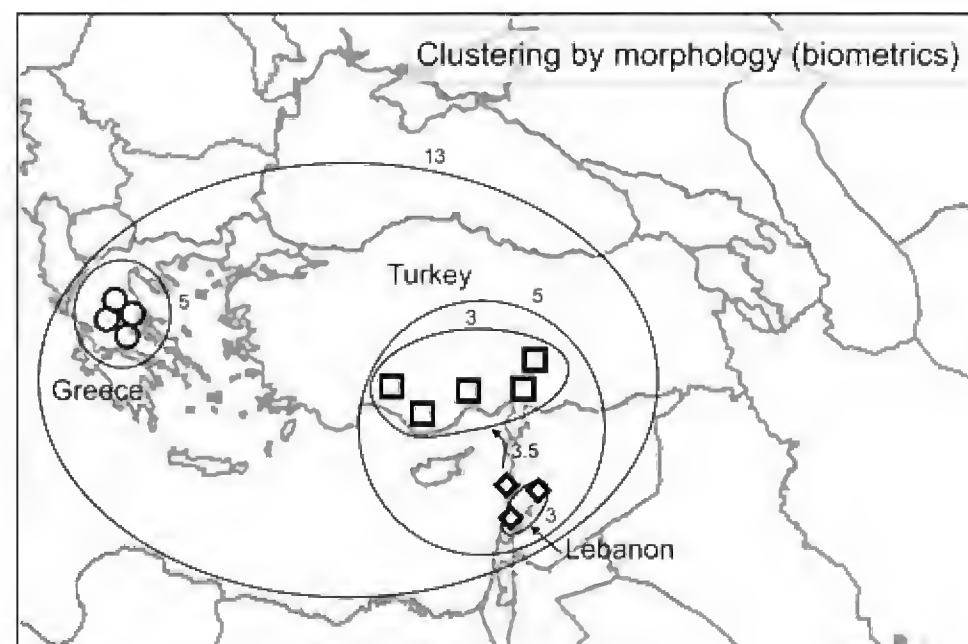


Figure 1. Clustering of *J. drupacea* populations by morphological data (adapted from Sobierajska et al. (2016)).

Juniperus drupacea is ancestral in the phylogeny of *Juniperus* (Adams, 2014, Adams and Schwarzbach, 2013). Adams (1997) reported the composition of the volatile leaf oil of *J. drupacea* was low in α -pinene in Greece (3.5-5.1%) but high in Turkey (14.3%) and the Crimea (cultivated) (22.1%) and high in limonene (+ β -phellandrene) in Greece (46.7 - 48.4 %), Turkey (55.6%) and Crimea (50.3%). *Juniperus drupacea* oil was high, but variable in δ -3-carene (7.0 - 22.3%). The analysis by Adams (1997) based on a few samples from Greece and literature reports from Turkey and Crimea.

The purpose of the present study was to present a more complete report on the composition of the volatile leaf oil of *J. drupacea* from Greece, Turkey and Lebanon, utilizing additional samples, all of which were analyzed in the same lab. In addition, nrDNA and cp DNA sequencing was performed to determine if the differentiation reported by Sobierajska et al. (2016) is discernable in DNA sequencing (and in leaf volatile oils data).

METHODS

Specimens for DNA and volatile leaf oils were collected of *J. drupacea* from: Greece:

2-3m tall, strong central axis, common with *Quercus coccifera*. Female cones falling off. Pollination in Sept? On highway, 18 km E of Tripolis at Achladokampos Pass, 700m, Greece, Prov. Arkadia. 7 Aug. 1987, Coll: Robert P. Adams 5651,5652,

small trees to 3 m, female common in the Achladokampos Pass but all trees young. 18 km E. of Tripolis. Prov. Arkadia. Lat. 37° 31.44'N, Long. 22° 37.92'E., 560m, Greece, 29 Aug. 1999, Coll: Robert P. Adams 8795,

large trees to 10m x 0.5 m dbh, male, abundant, 7.3 km E of Kosmos. Parnonas Mts., Mt. Parnonas Ski lift, 37° 08.00'N, 22° 45.63'E, 870m, Greece, 29 Aug. 1999, Coll: Robert P. Adams 8796,

pyramidal trees to 3-4m tall, strong central axis, common with *Quercus coccifera*. female cones falling off. Pollination in Sept? some trees monocious. On highway 7, 18km E of Tripoli at Achladokampos Pass, Country: Greece, Prov. Arkadia, 37° 31' 25" N, 22° 38' 19" E., 600m, 21 May 2017, Coll. Robert P. Adams 15227-15236.

Israel:

Mt Hermon, 33.31° N, 35.77° E, 1700m, June 2015, Israel, Coll. Hagar V. Leschner sn, Lab Acc. Robert P. Adams 14580, 14581

Lebanon:

Ehmej, on the road up the mountain. 34° 07' 58" N, 35° 49' 04" E, 1350 m, 22 Sept 2015, Lebanon, Coll. *Madga Bou Dagher-Kharrat, Ehmej 1-5* (=LEB-2), Lab Acc: *Robert P. Adams 14675-14679*;
 Mrebbine, Sir Danniyeh, 34° 24' 29" N, 36° 05' 13" E, 1400 m, 20 Sept 2015, Lebanon, Coll. *Madga Bou Dagher-Kharrat, Mrebbine 1-5* (=LEB-3, ~Dufram), Lab Acc: *Robert P. Adams 14680-14684*.
 Mchati Keserwan, 34° 02' 02" N, 35° 45' 53" E, 1180 m, 22 Sept 2015, Lebanon, Coll. *Madga Bou Dagher-Kharrat, Mchati Keserwan 1-5* (=LEB-1), Lab Acc: *Robert P. Adams 14685-14689*

Turkey:
 Camliyayla (Namrun), above Kole village, vicinity of Hotel Dag, 37° 10' 6.41" N, 34° 36' 3.8" W., 1250 m, Jan. 2017; Turkey, Coll. *S. Tugrul Koruklu sn*, Lab Acc. *Robert P. Adams 15220*;
 Alanya, Derekoy, Gedevet Pasture, Park Orman. 36° 39' N, 32° 10' E, elev. ca 1200 m, 19 Sept 2015, Prov. Antalya: Turkey, Coll. *Tugrul Mataraci & Sezai Vzun 2015-31*, Lab Acc: *Robert P. Adams 14693*;
 Gedevet Oastyrem Derince, on road to Sapacitlik, in the river bed. 36° 38' 23.27" N, 32° 03' 38.44" E., 1036 m, 13 Sep. 2017, Prov. Antalya, Turkey, Coll. *T. Mataraci, O. Demir & B. Cingay 8332*, Lab Acc. *Robert P. Adams 15307*
 Gedevet Oastyrem Derince, on road between Antalya and Askeki county, 30 km, 37° 03' 32.85" N, 31° 44' 47.34" E, 1132m, 12 Sep. 2017, Prov. Antalya, Turkey, Coll. *T. Mataraci, O. Demir & B. Cingay BCNG 8328A*, Lab Acc. *Robert P. Adams 15308*

Voucher specimens are deposited in the herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM), D (maldehy) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R7 (Biomatters, available from <http://www.geneious.com/>), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

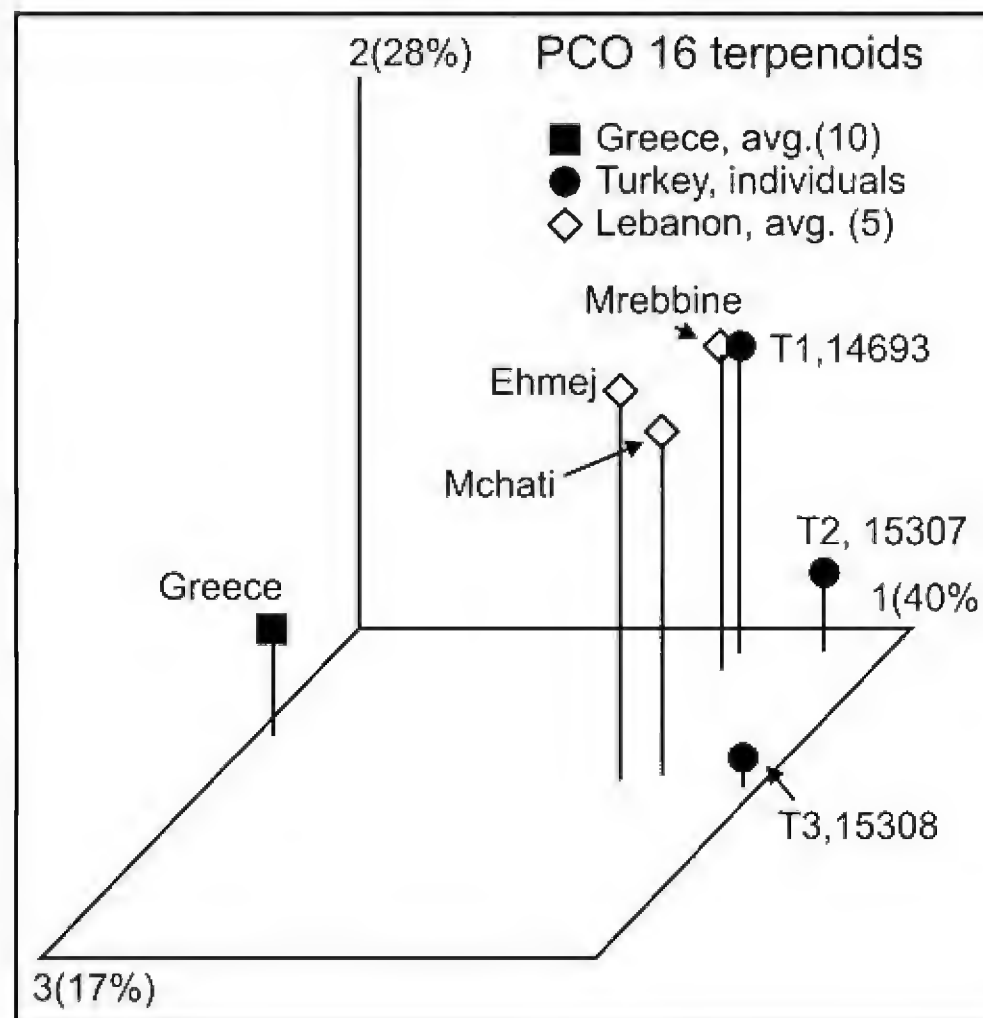
The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on

an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS

The composition of the leaf essential oils from Greece (avg. 10 plants), Turkey (3 plants), and three locations in Lebanon: Leb-Mb (avg. 5 plants), Leb-Mc (avg. 5 plants), and Leb-Eh (avg. 5 plants) are given in Table 1. The leaf oils of the Turkey/ Lebanon plants contained one unique terpene (trans-verbenol, 0.1-1.4%) that was absent in the Greece plants (Table 1). The Greece oil contained three terpenes not found in the Lebanon/ Turkey plants: (ar)-curcumene (2.2%), β -alaskene (0.3%) and α -alaskene (0.4%) (Table 1). Four other terpenes were in higher concentration in the Greece oils: camphene (0.4%), δ -3-carene (10.9%), p-mentha-1,5-dien-8-ol, isomer (0.3%) and 4-terpineol (0.3%). Three terpenes were higher (Table 1) in Turkey and Lebanon oils: α -pinene (10.5 - 32.9%), hexadecanoic acid (0.4 - 1.4%) and trans-totarol (0.3 - 1.2%).

Factoring the similarity matrix (based on quantitative comparisons of sixteen major compounds), resulting in three eigenroots that accounted for 84% of the variance among OTUs. The eigenroots appeared to asymptote after the third eigenroot. Principle Coordinate Ordination (PCO) analysis of the similarity matrix revealed the major trend (coordinate 1, 40%, Fig. 2) was the differentiation of the Greece oil from the oils of Turkey and Lebanon (Fig. 2).



The second coordinate (28%) separated Lebanon (Ehmej, Mchati, Mrebbine) and T1 (Turkey, 14693) from two other Turkey individuals (T2, T3, Fig. 2).

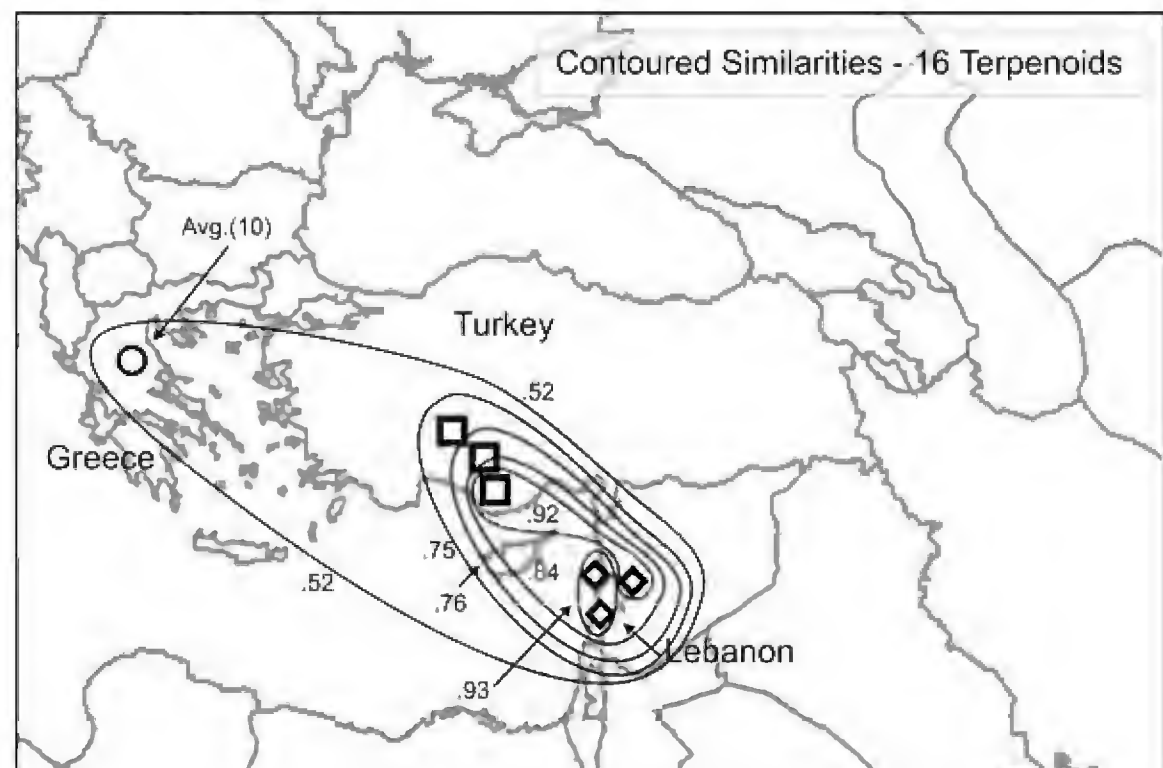
Figure 2. PCO of Greece population (avg. 10), three Lebanon populations (5 each, Ehmej, Mchati, Mrebbine) and three Turkey plants (T1, T2, T3).

The third coordinate (17%) separated T2 from T3 and (Eh, Mc) from (Mb, T1, Fig. 2).

Plotting contoured similarity clustering on a geographic map gives one the spatial aspect of the variation (Fig. 3). Notice that all the Turkey-Lebanon oils cluster at a 0.75 similarity (Fig. 3), whereas the Greece oil is only admitted into the final cluster at a similarity of 0.52. This shows the magnitude (considerable) of differentiation between Greece and Turkey-Lebanon oils. In general, the clustering is similar to that of the morphology (cf. Fig. 1 vs. Fig. 3).

It is also interesting that two of the Lebanon populations (Eh, Mc) cluster at 0.93, as shown by their nearly identical percent concentrations of components in Table 1. One Lebanon population (Mb) clusters with a Turkey plant (T1) at 0.92 (also notice the similarity in their oils in Table 1).

Figure 3. Contoured clustering based on 16 major terpenoids. See text for discussion.



It is informative to compare the morphological clustering (Sobierajska et al. 2016) versus clustering using 16 terpenoids (Fig. 4). Both morphology and terpenoids show major differentiation between Greece (Europe) and Asia plants. Both data show an individual from Lebanon clustering with a plant from Turkey (Morphology: Leb-2, TAT-1; Terpenoids: T1, LEB3-Mrebbine, Fig. 4).

Among the Turkey plants, morphology displays less diversity than is seen in terpenoids (Fig. 4). Otherwise, the clustering is very similar between the morphology and terpenoids data sets.

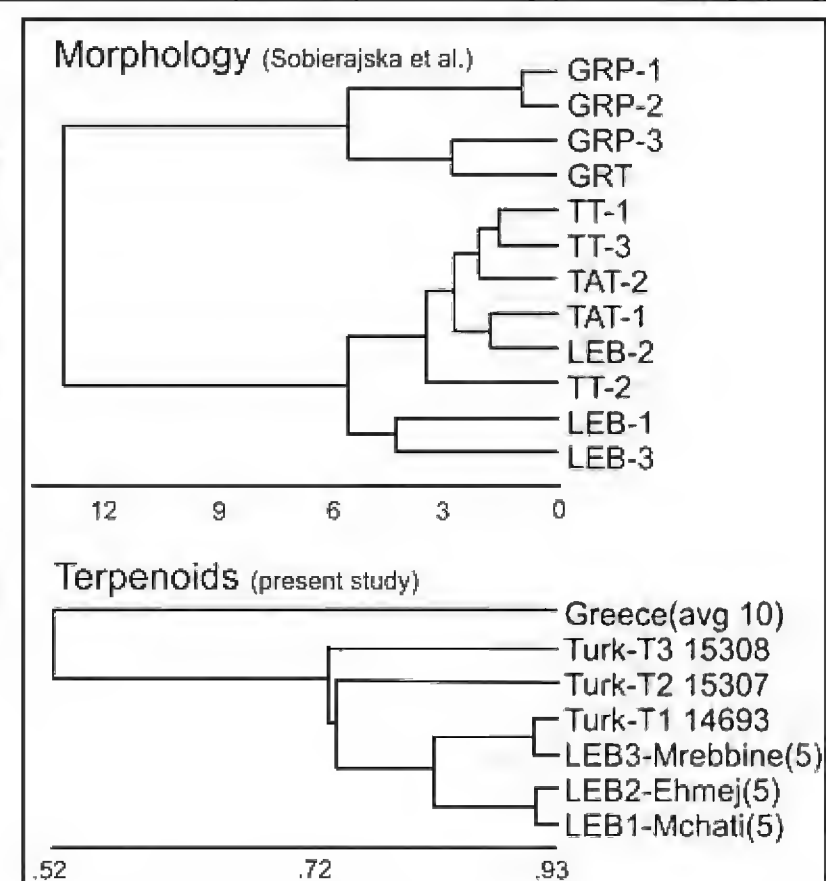


Figure 4. Comparison of clustering based on morphology (Sobierajska et al. 2016) and terpenoids (this study).

In addition to the analysis of terpenoids, we also sequenced nrDNA (ITS) and four cp regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF. These gene regions produced 3,423 bp of data. Only one SNP was found (in nrDNA) that separated Greece from Turkey-Lebanon-Israel. No informative SNPs were found in petN-psbM, trnS-trnG, trnD-trnT or trnL-trnF cp regions.

A Bayesian tree shows (Fig. 5) *J. drupacea* (monotypic in sect. *Caryocedrus*) well resolved from *Juniperus* sect *Juniperus* as previously shown (Adams, 2014, Adams and Schwarzbach, 2013). All the Greece plants group together and that clade is separated by one SNP in the nrDNA region. One SNP (in nrDNA) resolved the clade containing: Israel, 14580; Mc Leb, 14685 and Mc Leb, 14686. Eh Leb, 14686 contained a unique SNP, but otherwise there was no variation found. Although the divergence of the Greece plants was based on only one SNP, it was consistent among the seven plants and supports the morphology, nSSR, and terpenoids in showing differentiation between Greece (Europe) and Asia plants.

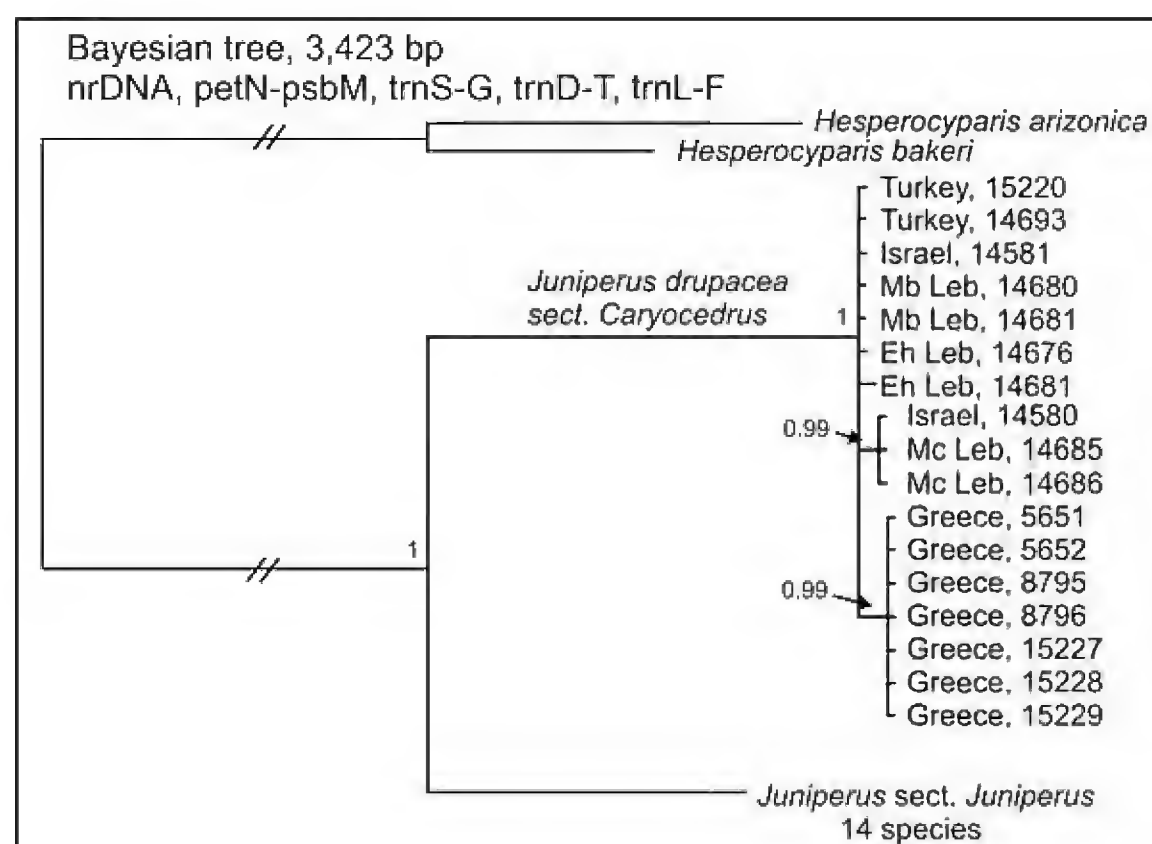


Figure 5. Bayesian tree based on 3,423 bp, nrDNA, petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF. Numbers at the branch points are posterior probabilities.

This study reconfirms the work of Sobierajska et al. (2016) that differentiation has occurred between Europe (Greece) and Asia (Turkey and Lebanon) in *J. drupacea*. Although the differences do not support the recognition of a new variety or subspecies, they present significant evidence that supports the thesis that the Aegean Sea presented a significant barrier during the Pleistocene to gene flow between Europe and Asia populations of *J. drupacea* and has contributed to the differentiation documented in this study and that of Sobierajska et al. (2016).

A hypothetical ancestor of the species, *Juniperus drupacea-pliocenica* Rer., probably diverged prior to 30–35 Mya (Mao et al., 2010) and evolved on the plate of the European continent (Palamarev, 1989; Kvaček, 2002; Palamarev, Bozukov, Uzunova, Petkova, Kitanov 2005). The colonization of terrains of the contemporary Peloponnese, Anatolia and the north-western part of the Arabian plateau might have occurred during Oligocene and the early Miocene (Burdigalian, 20.44–15.97 Mya), when a solid land connection supporting plant migrations was formed between these regions (Rögl, 1999; Meulenkaamp & Sissingh, 2003; Popov et al., 2006). Subsequently, the separation of the Peloponnese microplate from Anatolia (Tortonian 11.60–27.24 Mya) created the Aegean Sea. This presented a barrier, triggering their differentiation, afterward magnified during Messinian salinity crisis. The geographic range of *J. drupacea* was reduced during Pliocene climate cooling and drying (Thompson, 2005). These climate changes probably caused altitudinal migration, resulting in spatial isolation between populations inhabiting particular mountain massifs and, consequently, in subsequent genetic divergence (Sobierajska et al. 2016). The very dynamic climate fluctuations during Pleistocene, with cold (glacial) and hot (interglacial) periods, additionally modified by humid and/or dry phases (Roberts et al. 2011; Dean et al. 2015) influenced geographic distribution of organisms, including *J. drupacea*. These factors seem likely responsible for the current geographic range and at least partly for genetic, biochemical and morphological differentiation in populations of *J. drupacea*.

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Table 1. Leaf essential oil compositions for *Juniperus drupacea* from Greece, Turkey and Lebanon.

921	Tricyclene	t	t	t	t	t	t	t
924	α -thujene	t	t	t	t	t	t	t
932	α-pinene	5.0	10.5	16.6	12.5	12.3	32.9	32.8
945	α -fenchene	t	t	t	0.1	t	t	t
946	Camphene	0.4	t	t	0.1	t	0.2	0.2
969	Sabinene	0.2	t	t	0.2	0.2	0.2	0.2
974	β -pinene	0.3	0.9	0.7	1.0	0.8	1.8	1.5
988	Myrcene	2.3	3.2	2.7	3.1	2.6	3.2	3.0
1002	α -phellandrene	t	0.2	t	0.1	t	t	t
1008	δ-3-carene	10.9	-	1.2	0.7	0.4	t	t
1014	α -terpinene	t	t	t	t	t	t	t
1020	p-cymene	0.2	t	0.5	0.1	t	t	t
1024	Limonene	31.3	26.2	17.4	34.6	32.0	21.5	21.3
1025	β-phellandrene	28.1	26.2	17.4	34.7	31.2	21.5	21.3
1054	γ -terpinene	0.1	t	t	0.1	t	t	t
1086	Terpinolene	1.1	0.3	0.6	0.4	0.4	0.7	0.5
1099	Linalool	t	0.2	0.8	0.1	0.1	0.2	0.2
1100	n-nonanone	-	t	t	t	t	t	t
1118	cis-p-mentha-2-en-1-ol	t	t	t	t	t	-	-
1123	α -camphenal	t	0.1	0.5	-	0.2	0.4	0.5
1132	cis-limonene oxide	0.1	-	-	-	-	-	-
1135	trans-pinocarveol	-	t	0.9	-	0.2	0.6	0.7
1136	trans-p-mentha-2-en-1-ol	-	0.1	t	0.1	-	-	-
1139	trans-limonene oxide	-	-	-	-	-	-	-
1140	trans-verbenol	-	0.2	1.4	0.1	0.4	0.7	0.8
1141	Camphor	t	-	-	-	-	-	-
1156	p-mentha-1,5-dien-8-ol, isomer	0.3	t	t	t	t	0.1	t
1166	p-mentha-1,5-dien-8-ol	0.2	t	t	t	0.1	0.2	0.2
1174	terpinen-4-ol	0.3	0.1	t	0.1	0.1	0.1	0.2
1176	m-cymen-8-ol	0.1	t	t	t	t	t	t
1179	p-cymen-8-ol	0.1	t	t	t	t	t	t
1186	α-terpineol	0.4	0.3	0.5	0.2	0.1	0.1	0.1
1191	cis-dihydrocarvone	t	-	-	-	-	-	-
1195	Myrtenol	t	t	0.2	t	t	0.1	0.2
1195	cis-piperitol	t	-	-	-	-	-	-
1204	trans-dihydrocarvone	-	-	-	-	-	-	-
1204	Verbenone	0.1	t	0.6	t	0.1	0.2	0.2
1215	trans-carveol	0.1	t	0.3	t	0.3	0.2	0.3
1223	Citronellol	t	-	-	-	-	-	-
1226	cis-carveol	t	-	-	-	-	-	-
1239	Carvone	0.1	0.2	0.3	0.1	0.1	t	t
1241	carvacrol, methyl ether	-	t	t	t	0.1	t	t
1244	car-3-en-2-one	t	-	-	-	-	-	-
1249	Piperitone	t	-	-	-	-	-	-
1287	bornyl acetate	t	t	t	t	t	t	t
1345	α -cubebene	0.5	t	0.4	0.1	0.2	0.2	0.2
1374	α -copaene	0.5	t	0.7	0.1	0.4	0.4	0.5
1387	β -bourbonene	t	t	t	t	t	t	t
1417	(E)-caryophyllene	0.4	0.6	1.2	0.2	0.9	0.9	0.9
1429	cis-thujopsene	t	-	-	-	-	-	-
1448	cis-muurola-3,5-diene	t	t	t	t	-	-	-
1452	α -humulene	t	0.5	0.7	0.2	0.4	0.4	0.4
1465	cis-muurola-4(14),5-diene	0.3	0.5	0.5	0.2	t	t	t
1478	γ -muurolene	t	0.7	1.4	0.1	0.3	0.3	0.3
1480	germacrene D	1.5	2.9	3.5	1.5	1.9	1.9	2.1
1480	(ar)-curcumene	2.2	-	-	-	-	-	-
1496	Valencene	-	0.3	0.4	t	0.1	t	0.1
1498	β-alaskene	0.3	-	-	-	-	-	-
1500	α -muurolene	0.2	t	0.4	t	0.2	0.2	0.2
1505	(E,E)- α -farnesene	-	-	-	-	0.2	t	t
1512	α -alaskene	0.4	-	-	-	-	-	-
1513	γ -cadinene	0.6	1.3	1.8	t	1.1	0.6	0.8
1521	trans-calamenene	0.4	t	0.7	0.1	0.1	0.1	0.2
1522	δ -cadinene	t	t	0.7	0.1	0.1	0.2	0.3

KI	Compound	Greece avg.	Turk-T2 15307	Turk-T3 15308	Turk-T1 14693	Leb-Mb 14691	Leb-Mc 14692	Leb-Eh 14690
1533	10-epi-cubenol	0.2	0.4	0.5	0.1	0.2	0.1	0.1
1533	trans-cadina-1,4-diene	0.3	0.4	0.5	0.1	0.2	0.1	0.2
1561	(E)-nerolidol	-	0.1	0.4	0.2	0.1	-	t
1570	Dendrolasin	-	0.1	0.1	t	-	-	-
1582	(ar-) tumerol	t	-	-	-	-	-	-
1582	caryophyllene oxide	0.3	1.0	1.5	t	0.7	0.6	0.6
1586	germacra-4(15),5,10(14)- trien-1-al, isomer I	t	0.5	0.5	t	0.3	0.2	0.2
1594	salvial-4(14)-en-1-one	t	0.5	0.7	t	0.3	0.2	0.2
1608	humulene epoxide II	0.4	0.5	0.8	t	0.3	0.2	0.3
1610	salvial-4(14)-en-1-one, isomer I	0.6	0.9	1.2	0.1	0.3	0.2	0.2
1638	epi- α -cadinol	0.2	0.3	0.4	0.1	0.3	0.2	0.3
1640	epi- α -muurolol	0.2	0.3	0.3	0.1	0.3	0.2	0.3
1652	α -cadinol	1.1	1.8	2.1	0.7	0.8	0.6	0.7
1685	germacra-4(15),5,10(14)- trien-1-al	0.3	0.7	0.5	0.2	0.4	0.3	0.3
1687	eudesma-4(15),7-dien-1- β -ol	0.4	0.7	0.6	0.3	0.4	0.3	0.3
1711	Pentadecanal	t	t	0.4	t	t	t	t
1840	hexahydrofarnesyl acetone	-	t	t	t	t	t	t
1959	hexadecanoic acid	t	1.4	1.3	0.7	0.4	0.5	0.4
1987	manoyl oxide	1.3	2.5	4.5	0.3	4.0	1.9	0.8
2055	Abietatriene	t	0.5	0.5	0.2	0.1	0.3	t
2105	Isoabienol	0.3	1.3	2.1	0.6	0.7	0.8	0.5
2112	phytol, isomer I	0.2	1.3	0.8	0.4	0.4	0.6	0.3
2231	methyl communate, isomer I	-	t	t	t	0.1	0.3	0.2
2314	trans-totarol	t	0.3	1.2	0.3	0.3	0.5	0.3
2331	trans-ferruginol	t	0.5	0.6	0.2	t	0.2	t

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.